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ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
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SAM 605

9 CFR 113.104(e)	May 25, 1982 Supersedes November 1, 1974	Erysipelothrix rhusiopathiae Agent

SUPPLEMENTAL ASSAY METHOD FOR POTENCY TESTING OF ERYSIPELAS BACTERINS IN SWINE

A. SUMMARY

This is a method for determining the potency of an Erysipelas bacterin, as set forth in 9 CFR 113.104(e). It is a test to determine the immunity of vaccinated swine by challenging with a virulent culture of Erysipelothrix rhusiopathiae.

B. MATERIALS

1. Animals - pigs, susceptible to erysipelas, 20-30 pounds.

2. Media and Diluent

a. One percent Peptone in Soil Buffer solution

Peptone	10.00 grams
Sodium Phosphate Dibasic (anhydrous)	12.02 grams
Potassium Phosphate Monobasic	2.09 grams
Distilled Water	1000.00 ml.

All ingredients are mixed thoroughly in a 2000 ml Erlenmeyer flask. The pH is adjusted to 7.5. Ninety-nine ml amounts are dispensed in milk dilution bottles with screw caps and sterilized by autoclaving for 15 minutes at 121°C.

b. Erysipelas Challenge Culture Media

The infusion is prepared as follows:

Horse Meat (no fat)	454 grams
Horse Liver	18 grams
Distilled Water	1000 ml

The meat and liver are thawed (if frozen) and fat is trimmed off. It is ground and dispersed in the hot distilled water in a stainless steel cooker with a spigot at the bottom. The infusion is heated to boiling, simmered (just below the boiling point) for one hour and then brought back to a boil for 3 to 5 minutes. The infusion is allowed to cool and settle

for at least two hours. The broth is drawn off through the spigot by gravity and filtered through No. 2 Whatman filter paper. The following ingredients are added per liter of filtered broth:

Sodium Phosphate Dibasic (anhydrous)	11 grams
Potassium Phosphate Monobasic	1 gram
Peptone	20 grams
Gelatin - granular	5 grams
Ox Bile (if frozen, thaw)	10 ml

The medium is adjusted to pH 8 with 10N NaOH before sterilization. The medium is sterilized by filtering through a Model 7B Hormann filter (filter grades D5 and D9). Ninety-nine ml of medium are dispensed into 160 ml milk dilution bottles. The final pH is adjusted to 7.6 to 7.8.

c. 5% Bovine Blood agar - plates.

d. Normal horse serum - sterile.

3. Test Materials

a. Challenge material - Erysipelothrix rhusiopathiae - Strain E 1-6.

b. Sample(s) of Erysipelothrix rhusiopathiae bacterin(s) to be tested.

4. Equipment (Sterile)

a. Serum bottle - 50 ml.

b. Spectrophotometer (e.g., Bausch and Lomb, Spec 70).

c. Syringes - 3 ml plastic disposable.

d. Needles - 20 gauge (1 inch).

e. Pro-Pipet.

f. Pipettes - 1 ml, 2 ml and 10 ml.

g. Test tubes - 13 x 75 screw cap (for spec) and 16 x 125 screw cap (for dilutions).

C. PROCEDURES

1. Upon receipt of samples a test series is assigned and worksheets are prepared with the following information: test series number, serial number, bacterin dose, route of vaccination, identity of challenge culture, dose of challenge culture, date of vaccination, challenge and termination, sex, color, and ear tag identification of swine, and the initials of the person conducting the test.

2. The test animals are received and observed for 1 week prior to initiation of test. The pigs are identified by ear tags and grouped by random selection. The temperature of each pig is taken for 3 days prior to vaccination to establish a normal range.

3. Four susceptible pigs are vaccinated with the bacterin to be tested according to the directions on the label with one recommended swine dose.

Four (4) susceptible pigs from the same source are randomly selected for controls. These animals are observed daily for abnormal reactions. Temperatures are taken three times a week prior to challenge to establish a normal range.

4. Preparation of the Challenge Inoculum

   a. Eighteen to twenty hours prior to time of challenge, each of two vials of Strain E 1-6 challenge culture is reconstituted with 1.5 ml of 1% peptone in soil buffer solution and mixed well. Ten (10) ml of sterile normal horse serum are added aseptically to each of three milk dilution bottles containing 90 ml of Erysipelas media (B.,2.,b.). Two of the three bottles are each inoculated with the entire volume of each seed vial. The third bottle is held as an uninoculated control. All three bottles are incubated at 37°C.

   b. An 18-20 hour culture is adjusted to 40% LT at 600 nm on a spectrophotometer. Tenfold dilutions are made of the 40% culture.

5. Fourteen to twenty-one days following vaccination, all test animals are challenged intramuscularly with 2 ml of the challenge dilution (e.g., 10^{-5}). Bacterial count of the challenge culture is done on 5% B.A. media.

D. OBSERVATION OF SWINE AFTER CHALLENGE

1. The swine are observed daily for clinical signs as described in 9 CFR 113.104(e) and temperatures taken for 7 days. This information is recorded on the worksheets.

E. INTERPRETATION

The results are interpreted in accordance with 9 CFR 113.104(e).